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Human Monoamine Oxidase A Gene Determines Levels of Enzyme Activity

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Summary

Monoamine oxidase (MAO) is a critical enzyme in the degradative deamination of biogenic amines throughout the body. Two biochemically distinct forms of the enzyme, A and B, are encoded in separate genes on the human X chromosome. In these studies we investigated the role of the structural gene for MAO-A in determining levels of activity in humans, as measured in cultured skin fibroblasts. The coding sequence of the mRNA for MAO-A was determined by first-strand cDNA synthesis, PCR amplification, and direct dideoxy sequencing. Two single-basepair substitutions were observed in cDNAs from cells with a 30-fold difference in activity levels. These two substitutions were in the third base of a triplet codon and hence did not affect the deduced amino acid sequence but did affect the presence or absence of restriction-enzyme sites for *Eco*RV and *Fnu*4HI, which could be elucidated on PCR fragments derived from genomic DNA or cDNAs. A third polymorphism for *Msp*I in the noncoding region of the *MAOA* gene was also evaluated by Southern blot analysis using genomic DNA. Statistically significant associations were observed between the alleles for *MAOA* and levels of MAO activity in human male fibroblast lines. This association indicates that the *MAOA* gene itself is a major determinant of activity levels, apparently, in part, through noncoding, regulatory elements.

Introduction

Monoamine oxidase (MAO; E.C.1.4.3.4) is a flavin-containing enzyme which degrades a variety of biogenic amines, including the neurotransmitters norepinephrine, dopamine, and serotonin (for review, see Weyler et al. 1990). Two forms of the enzyme, MAO-A and MAO-B, have been identified on the basis of the difference in molecular weight, substrate affinities, inhibitor sensitivities, and immunological properties. These enzymes are expressed throughout the body but differ in developmental and cell-specific expression. In human brain the predominant form is MAO-B (Garrick and Murphy 1982), expressed at highest levels in astrocytes and serotonergic neurons, while MAO-A is expressed at highest levels in catecholaminergic neurons (Fowler et al. 1987; Thorpe et al. 1987). A similar neural distribution has been observed in rats (Levitt et al. 1982) and nonhuman primates (Westlund et al. 1985). Recently, full-length cDNA clones for human MAO-A and MAO-B have been characterized (Bach et al. 1988; Hsu et al. 1988). The nucleotide and amino acid sequences for human MAO-A and MAO-B (Bach et al. 1988; Hsu et al. 1988), as well as for bovine MAO-A and MAO-B (Powell et al. 1989), indicate that these two proteins are encoded in separate genes (Hsu et al. 1989). Genes for MAO-A and MAO-B have been mapped to the p11.23–11.4 region of the human X chromosome (Ozelius et al. 1988; Lan et al. 1989; Levy et al. 1989).

In peripheral tissues accessible from humans, MAO-A can be measured in cultured skin fibroblasts (Breakefield et al. 1976) and placenta (Weyler and Salach 1985), and MAO-B can be measured in platelets (Donnelly and Murphy 1977) and lymphocytes (Bond and Cundall 1977). Levels of total MAO activity in skin fibroblasts are quite low, with proportions of MAO-A activity and MAO-B activity being 80%–

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100% and 0%–20%, respectively (Edelstein et al. 1978). Values measured in fibroblasts from MZ twins are highly correlated, and levels in individual lines are stable from subculture to subculture under controlled conditions of growth, indicating a strong genetic determinant (Breakefield et al. 1980). Levels of MAO-A activity vary over 50-fold among control individuals. Dramatic increases in MAO-A activity in these cells can be seen as a result of "aging," due both to donor age and entrance of cells into senescence in culture and to treatment with glucocorticoids (Breakefield et al. 1980; Edelstein and Breakefield 1986). Stable levels of MAO-B activity also vary over 50-fold in the normal population (Murphy et al. 1976) and are genetically determined (Gershon et al. 1980). A single major locus appears to be the main determinant of MAO-B activity (Rice et al. 1984).

Variations in MAO activity appear to affect neurophysiologic and behavioral traits in humans and animals. Pharmacologic inhibition of activity in rodents leads to an accumulation of catecholamines and serotonin in the brain and alters neuronal firing patterns causing autonomic dysfunction, seizures, motoric hyperactivity, and stereotypy (Campbell et al. 1979). In humans, moderate inhibition of MAO activity can lead to mood elevation, loss of REM sleep, motoric hyperactivity, orthostatic hypotension, and hyperreflexia without spasticity (Kupfer and Bowers 1972; Squires 1978; Murphy and Kalin 1980; Murphy et al. 1983).

A number of studies have documented a statistically significant association of low-normal levels of MAO-B activity with several psychiatric conditions, including schizophrenia, alcoholism, and paranoia (Murphy et al. 1977; Tabakoff et al. 1988; Sullivan et al. 1990). Recently, loss of *MAOA* and *MAOB* genes (Lan et al. 1989; Sims et al. 1989*a*) and total deficiency of MAO-A and MAO-B activities (Sims et al. 1989*a*, 1989*b*) have been demonstrated in males with atypical Norrie disease, who carry a submicroscopic deletion in the Xp11 chromosomal region (Diergaarde et al. 1989). These MAO-deficient patients have severe neurologic dysfunctions, including atonic and myoclonic seizures, microcephaly, autonomic dysfunction, sleep disturbance, profound mental retardation, and small stature (de la Chapelle et al. 1985; Gal et al. 1986; Donnai et al. 1988; Sims et al. 1989*a*), as well as marked changes in amine and amine metabolites (Murphy et al. 1990, 1991). In contrast, in classic Norrie patients (Warburg 1975), chromosomal deletions have not been described, and MAO activities and amine metabolites are normal (Sims et al. 1989*b*; Murphy et al. 1990).

There has not been an extensive evaluation of MAO-A activity in human disease states. In order to facilitate this evaluation, we investigated allelic variations in the *MAOA* gene at the mRNA and genomic DNA levels and correlated allele status with activity levels measured in fibroblasts.

Material and Methods

Oligonucleotides

Oligonucleotide primers, derived from the published sequence of human MAO-A cDNA (Bach et al. 1988; Hsu et al. 1988), were synthesized on a Milligen-BioSearch/Cyclone DNA synthesizer and purified by PAGE. Oligo $(dT)_{12-18}$ was purchased from Bethesda Research Laboratories (Gaithersburg, MD); dNTPs and ddNTPs were from Boehringer Mannheim (Indianapolis).

Cell Lines

Control human fibroblast lines (prefixed with HF) were established from skin biopsies of the inner forearm or abdomen from control males in our laboratory. Other control lines were obtained from the American Type Culture Collection, Bethesda, MD (names designated by letters only); from the Institute for Medical Research, Camden, NJ (prefixed with GM); and from Dr. Samuel Goldstein, McMaster University, Hamilton, Ontario (A1, A2, and R-E11). Lines from Lesch-Nyhan patients were obtained as follows: LN Bur from Dr. Uta Francke, Yale University School of Medicine, New Haven, CT; 115 and 87 from Dr. Roy Breg, Yale University School of Medicine; GM152, GM537, GM1906, GM1662, and GM2227 from the Camden Cell Repository, Camden, NJ; On Ser, To Ser, and Sal Mat from the American Type Culture Collection, Bethesda, MD; and S2 from Dr. J. Edwin Seegmiller, University of California School of Medicine, La Jolla, CA. The Lesch-Nyhan syndrome does not affect MAO activity in fibroblasts (Costa et al. 1980). All cell lines were studied in the proliferative stage of growth in DMEM (GIBCO) with 10% FCS (GIBCO) (Edelstein et al. 1978). Lines were harvested 5–7 d after reaching confluency. In some cases cell were treated for 1–2 d with 50 nM dexamethasone

prior to harvesting, to increase levels of MAO-A mRNA (Sims et al. 1989*a*).

RNA Extraction and cDNA Synthesis

Monolayers on 2,150-cm^2 flasks (20–50 mg protein) were rinsed twice with PBS (GIBCO) and kept at 4°C. RNA was extracted using a cesium chloride extraction protocol (Chirgwin et al. 1979). The yield of total RNA was 10–30 μg/mg protein. First-strand cDNA synthesis was carried out in a reaction mix containing 10 μg total cellular RNA, 50 mM Tris (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 20 mg BSA/ml, 0.5 mM each of dATP, dGTP, dCTP, and dTTP, 2 μM oligo $(dT)_{12-18}$, 10 U human placental ribonuclease inhibitor (BRL), and 500 U M-MLV reverse transcriptase in a final volume of 50 μl (Newman et al. 1988). The reaction was incubated at 37°C for 60 min, and this was followed by phenol extraction and ethanol precipitation. The pellet was resuspended in 20 μl 1 × TE buffer, pH 8.0, and was stored at −20°C.

PCR Amplification and Sequencing

Two-microliter aliquots of the first-strand cDNA products from 10 μg total fibroblast RNA or 500 ng genomic DNA were incorporated in 50 μl of a reaction mix containing 50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM $MgCl_2$, 100 μg gelatin/ml, 1 mM of each dNTP, 200 nM of each primer, and 2.5 U of *Taq* DNA polymerase (Perkin Elmer—Cetus). Thirty cycles of amplification (94°C for 1 min, 57°C for 2 min, 72°C for 3 min) were carried out with a final extension time of 10 min (Saiki et al. 1988). Three microliters of reaction mix was electrophoresed in a 1.1% agarose gel and was visualized by staining with ethidium bromide by using *Bst*EII fragments of lambda DNA as size markers. The rest of the reaction mix was loaded on a 2% NuSieve GTG agarose (FMC Bioproducts) in 1 M Tris-borate/EDTA buffer (pH 8.0) and electrophoresed for 6 h at 4.4 V/cm; the bands were excised from the gel or were eluted onto DEAE membranes (Schleicher & Schuell), as described (Dretzen et al. 1981), and were stored at −20°C.

A two-stage sequencing method with *Taq* DNA polymerase, [alpha-^{32}P]d-CTP, and double-stranded DNA templates was used (Gorman and Steinberg 1989) with minor modifications. In some cases 200 ng DNA eluted from the agarose gels was incorporated into sequencing reactions using modified T7 DNA polymerase, Sequenase (United States Biochemical Corp.), and [alpha-^{35}S]d-ATP, as described (Sanger et al. 1977).

Detection of RFLPs by Using PCR-amplified DNA

The PCR-amplified DNA was extracted with chloroform, precipitated with EtOH, and resuspended in H_2O for RFLP analysis. Approximately 300 ng DNA was digested with restriction enzymes *Eco*RV or *Fnu*4HI (New England Biolabs, Beverly, MA). DNA fragments were electrophoresed through 1% standard (*Eco*RV) or 3% low-melting-temperature (FMC Bioproducts) plus 1% standard agarose gels (*Fnu*4HI) at 8.5 V/cm for 3 h. The gels were then stained with ethidium bromide, and DNA was visualized under UV light.

DNA Extraction and Southern Blot Analysis

Total genomic DNA was extracted from fibroblast homogenates, as described (Maniatis et al. 1989). Ten micrograms genomic DNA was digested to completion with 40 U restriction endonuclease *Msp*I (New England Biolabs). Electrophoresis, blotting, and hybridization to the 1,300-bp *Xba*I-*Eco*RI fragment of genomic clone A2R/D7 were carried out as described (Ozelius et al. 1988, 1989).

Statistical Methods

Statistical analysis of the relationship between the MAO-A alleles and enzyme activity levels was determined by breaking the sample into a 2 × 2 (allele × activity level) table and applying (1) χ^2 test, (2) Cochran-Mantel-Haenszel statistics for the odds ratios, and (3) Kendall Tau B correlation coefficients to determine the strength of associations. The SAS version V computer program (SAS Institute Inc, Cary, NC, 1985) was used for the analysis. To test the possible age effect on allele associations, we stratified the whole sample into young (age <20 years) and old (age ≥20 years) subgroups and repeated the previous analysis.

Results

Previous studies in our laboratory have shown that MAO activity levels are stable for individual fibroblast lines over serial passages during the proliferative stage of growth (Edelstein et al. 1978; Costa et al. 1980). Levels of activity in control males 1–20 years of age cover a wide range, 0.1–84.5 pmol/min/mg protein (fig. 1). In order to determine whether these activity

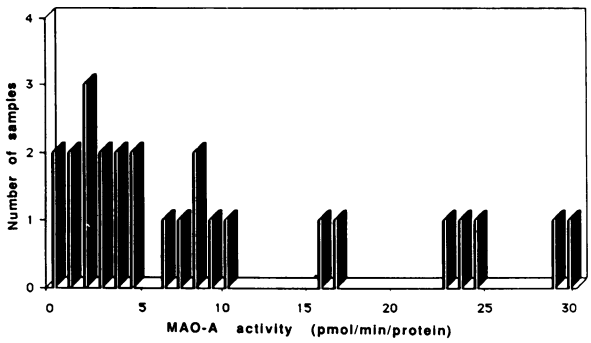

Figure 1 Distribution of MAO-A activity as measured in fibroblasts in control males under age 20 years. Activity measurements were done by using 30 μM tryptamine as substrate (Edelstein et al. 1978; Costa et al. 1980).

differences reflected variations in amino acid sequence, we analyzed the coding sequences of MAO-A cDNA from cell lines with low and high levels of MAO-A activity. Males were chosen for analysis since they have only a single allele for the MAO-A gene.

The full-length coding sequence (1.6 kb) for human MAO-A was amplified with two pairs of oligonucleotide primers designed to generate this cDNA in two overlapping fragments of 867 and 1,011 bp (fig. 2). Full-length coding sequences were determined for lines with low activity (GM500; 1 pmol/min/mg protein) and high activity (HF53; 27 pmol/min/mg protein);

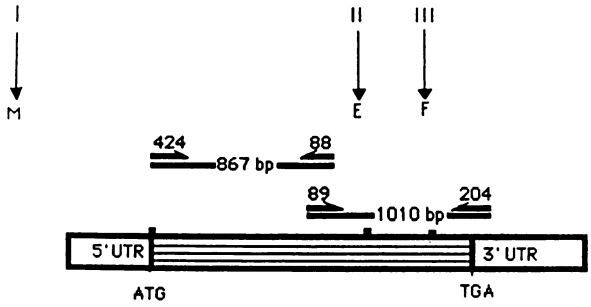

Figure 2 Schematic representation of human MAO-A cDNA and strategy used in amplification by PCR. 424 (5′-AAGAGAAGGCGAGTATCGC-3′), 88 (5′-TGGTTTCTCTCTGCTGGA-3′), 89 (5′-GACCTTGACTGCCAAGATT-3′), and 204 (5′-GATCACAAGGCTTTATTCTA-3′) are the oligonucleotide primers derived from the published sequence (Hsu et al. 1988). The cDNA was amplified with primer pairs 424–88 and 89–204, which generate 867- and 1,011-bp DNA fragments, respectively, and have a 53-bp overlap. The dashed part represents the translated region of the MAO-A gene that is 1.6 kb from the start codon ATG to the stop codon TGA. Arrows: I = *MSp*I polymorphism; II = T→G at 941; III = T→C at 1460. M = *Msp*I site; E = *Eco*RV site; F = *Fnu*4HI site; UTR = untranslated region.

partial sequences were determined for one with high activity (El San; 32 pmol/min/mg protein). The cDNA sequences of GM500 and HF53 were identical to the cDNA sequences published by Hsu et al. (1988), but two single-basepair differences were observed in El San. A G instead of a T was found at position 941 in El San, and a T instead of a C was found at position 1460. Both of these substitutions are in the third base of a codon and do not change the deduced amino acid sequence. However, these substitutions do affect restriction sites for the enzymes *Fnu*4HI (position 941) and *Eco*RV (position 1460). These differences also occur between the two published cDNA sequences for human MAO-A, hMAOA-7 (Bach et al. 1988) and HM11 (Hsu et al. 1988).

The allelic status of the cell lines was evaluated further by PCR amplification of cDNA fragments and digestion with *Fnu*4HI and *Eco*RV. The occurrence of a G at position 941 generates an additional *Fnu*4HI site in the amplified fragment. When the 1,011-bp fragment is digested with *Fnu*4HI, six fragments of estimated sizes 470, 250, 107, 78, 66, and 40 bp are observed for El San, and five fragments of 470, 250, 144, 107, and 40 bp are observed for GM500 and HF53 (fig. 3*A*). When a T is present at position 1460, two fragments of 420 and 590 bp are expected after *Eco*RV digestion of the amplified cDNA fragment, as seen for El San and not for GM500 and HF53 (fig. 3*B*).

We then evaluated the status of these two single-basepair substitutions at positions 941 and 1460 in 37 male lines with low and high MAO-A activity by restriction-digest analysis of PCR-amplified genomic DNA fragments. The *Eco*RV and *Fnu*4HI polymorphisms can be detected on 488- and 130-bp PCR-amplified genomic DNA fragments, respectively (figs. 4 and 5). The primers used to amplify these fragments are as follows (in 5′ and 3′ order): TTAAATGGTCTCGGGAAGG (sense orientation) and GCCCAATGACACAGCCTTT (antisense orientation) for the 488-bp genomic DNA fragment and GACCTTGACTGCCAAGAT (sense orientation) and CTTCTTCTTCCAGAAGGCC (antisense orientation) for the 130-bp genomic DNA fragment. A previously described *Msp*I polymorphism detected by genomic clone A2R/D7 (Ozelius et al. 1989) was also studied. The human *MAOA* gene is over 90 kb in length, and the genomic clone A2R/D7 contains the 5′ noncoding part of the gene (Chen et al., in press).

MAO-A activity levels and allele status for 40 males (ages 1–57 years) are shown in table 1. This sample

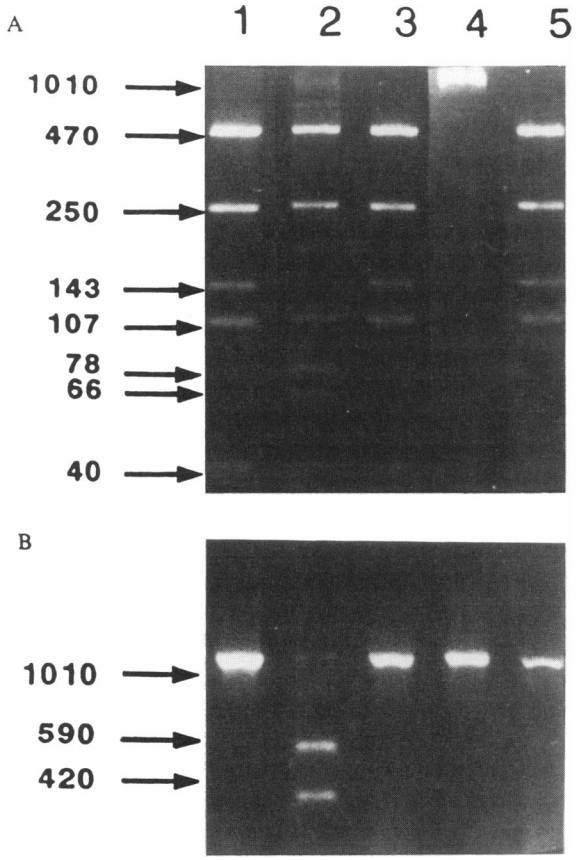

Figure 3 *A*, *Fnu*4HI polymorphism detected by restriction-digest analysis of 1,011-bp cDNA fragment amplified by PCR. Lanes 1 and 2, Cell lines HF53 and El San, respectively, with high activity and digested with *Fnu*4HI. Lane 3, Cell line GM500 with low activity and digested with *Fnu*4HI. Lane 4, MAO-A cDNA clone HM11 (Hsu et al. 1988) undigested. Lane 5, Clone HM11 digested with *Fnu*4HI. The presence of the *Fnu*4HI site was detected in line El San (lane 2), where the 143-bp fragment is replaced by 78- and 66-bp fragments. *B*, *Eco*RV polymorphism detected by restriction-digest analysis of the 1,011-bp cDNA fragment amplified by PCR. Lanes 1–3, DNA from cell lines HF53, El San, and GM500, respectively, digested with *Eco*RV. Lane 4, Undigested MAO-A cDNA clone HM11 (Hsu et al. 1988). Lane 5, Clone HM11 DNA digested with *Eco*RV. The *Eco*RV polymorphism was detected in cell line El San (lane 2).

group covers a range of activity levels, 0.1–179.2 pmol/min/mg protein. Two of the polymorphisms defined in this study (*Eco*RV and *Fnu*4HI) are in complete disequilibrium. Therefore these three polymorphisms define four possible haplotypes, only three of which were observed in this sample group. Haplotype A was found in 18/20 lines with enzyme activity levels <12 pmol/min/mg protein and in 9/20 lines with

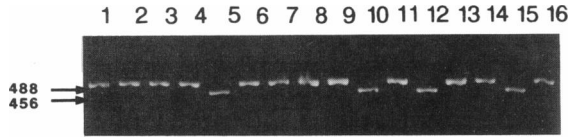

Figure 4 Representative gel showing *Eco*RV polymorphism detected by amplification of 488-bp fragment from genomic DNA and restriction-digest analysis. Lanes 1, GM537. Lane 2, GM497. Lane 3, A2. Lane 4, 115. Lane 5, GM1662. Lane 6, Rid Mor. Lane 7, HF17. Lane 8, HF9. Lane 9, GM2227. Lane 10, HF21. Lane 11, GM316. Lane 12, HF20. Lane 13, HF10. Lane 14, HF39. Lane 15, HF27. Lane 16, LN Bur. The 32-bp fragment ran out of the gel in lanes 5, 10, 12, and 15.

levels of ≥12 pmol/min/mg/protein. Haplotypes B and C were observed in 0/20 and 2/20, respectively, in the low-activity cell lines and in 3/20 and 8/20, respectively, in the high-activity lines. One of the two lines with low activity and a non-A haplotype was derived from a 1-year-old male, the youngest individual tested, and may reflect the donor age effect (see below). We employed the χ^2 test to determine the statistical significance of the allelic differences between high- and low-activity groups, and we used Cochran-Mantel-Haenszel statistics to determine the odds ratios. The comparison of these two groups revealed statistically significant differences in the distribution of these haplotypes in lines with low and high activity levels. The results of these statistical analyses are summarized. Significance of association between *Eco*RV, *Fnu*4HI, and *Msp*I alleles and activity levels was also determined by applying the Kendall Tau B test and was statistically significant, with correlation coefficients of .34641, .34641 and .48038 (corresponding to *P* values of .0305, .0305, and .0027), respectively.

The MAO activity levels measured in fibroblasts during the proliferative stage of growth appears to increase with the age of the donor (Breakefield et al. 1980) and must be considered in this evaluation. To minimize the age effect, the sample was divided into young (age <20 years) and old (age ≥20) subgroups.

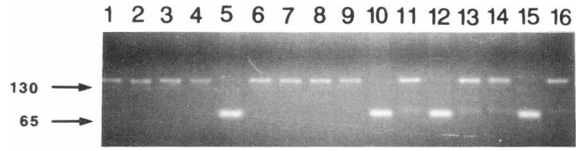

Figure 5 Representative gel showing *Fnu*4HI polymorphism detected by amplification of a 130-bp fragment from genomic DNA and restriction-digest analysis. The order of the lanes is as in fig. 5. The 65-bp fragment is a double band.

Table 1

Distribution of Alleles for MAO-A in Individuals with High and Low Enzyme Activity

Cell Line[a]	Age (years)	Mean ± SEM MAO Activity[b] (pmol/min/mg protein)	Haplotype[c]
HF56	17	.1 ± .1	A
GM537	12	.2 ± .1	A
GM409	7	.9 ± .2	A
GM497	4	.9 ± .7	A
On Ser	7	1.0 ± .2	A
GM2037	13	1.2 ± .6	A
A2	11	1.2 ± .6	A
GM500	10	1.4 ± .3	A
Sal Mat	14	1.6 ± .4	A
To Ser	9	2.2 ± .7	C
GM498	3	2.7 ± .7	A
GM152	9	3.5 ± .9	A
115	13	3.6 ± .3	A
GM323	11	5.4 ± .6	A
GM1906	15	6.0 ± 3.1	A
GM1662	1	6.2 ± 1.0	C
87	12	6.7 ± .7	A
A1	15	7.4 ± 1.1	A
Rid Mor	15	9.2 ± .8	A
HF17	33	10.6 ± 2.7	A
HF9	25	12.1 ± 4.2	B
GM2227	12	15.0 ± 3.2	B
S3	3	16.1 ± 2.2	C
HF46	36	16.5 ± 1.7	C
HF8	26	17.7 ± 4.2	C
HF45	25	20.0 ± 4.9	C
R-Ell	9	23.8 ± 3.3	B
GM316	12	24.5 ± 2.1	A
HF20	19	25.2 ± 3.2	C
HF53	36	26.7 ± 3.9	A
GM1653	37	27.1 ± 2.4	A
Ro Bel	14	31.7 ± 0.3	A
El San	8	31.9 ± 9.1	C
HF26	38	38.2 ± 2.1	C
HF10	23	41.8 ± 16.2	A
HF51	57	42.1 ± 10.4	A
HF39	54	52.3 ± 10.0	A
HF27	38	82.5 ± 16.0	C
HF52	47	84.5 ± 8.0	A
LN Bur	20	179.2 ± 30.9	A

[a] All skin fibroblast lines (except 115, GM537, On Ser, To Ser, Sal Mat, GM152, GM1906, S3, GM1662, GM2227, and LN Bur) are from control human males.

[b] Measured by a modification of the toluene extraction procedure of Wurtman and Axelrod (1963), as reported by Edelstein et al. (1978). Thirty micromoles [ethyl-^{3}H tryptamine HCl (25.5 Ci/mmol; NEN)] was used as substrate for MAO. Blank values were determined using clorgyline as inhibitor at a concentration of 10^{-4} M.

[c] Assigned with regard to the presence (+) or absence (−) of *Eco*RV, *Fnu*4HI, and *Msp*I restriction sites, respectively. A = (− − −); B = (− − +); C = (+ + +).

Thirteen of 14 "old" cell lines had high activity levels and hence could not be analyzed. Of the 26 individuals in the young subgroup, seven had high activity and 19 had low activity. Analysis of the young subgroup showed that statistical results for association between activity levels and *Eco*RV, *Fnu*4HI, and *Msp*I polymorphisms (table 2) were similar to those obtained for the total group (table 1), except that, in this case,

Table 2

Statistical Analysis of Distribution of Alleles for MAO-A in Individuals with High and Low Enzyme Activity

Group, Polymorphism, and Status	No. of Alleles with High Activity	No. of Alleles with Low Activity	P Value for χ^2 Test	Odds Ratio (95% confidence interval)
Whole ($n = 40$):				
*Eco*RV:				
+	8	2	.028	6.0 (1.08–33.27)
−	12	18		
*Fnu*4HI:				
+	8	2	.028	6.0 (1.08–33.27)
−	12	18		
*Msp*I:				
+	11	2	.002	11.0 (2.00–60.57)
−	9	18		
Haplotype:[a]				
A	9	18		
B	3	0		
C	8	2		
Young[b] subgroup ($n = 26$):				
*Eco*RV:				
+	3	2	.064	6.37 (.78–51.78)
−	4	17		
*Fnu*4HI:				
+	3	2	.064	6.37 (.78–51.78)
−	4	17		
*Msp*I:				
+	5	2	.002	21.25 (2.35–191.59)
−	2	17		
Haplotype:[a]				
A	2	17		
B	2	0		
C	3	2		

[a] A = (− − −); B = (− − +); C = (+ + +); (−) and (+) = absence and presence, respectively, of restriction sites for *Eco*RV, *Fnu*4HI, and *Msp*I.

[b] Those <20 years old. Only the young subgroup is shown, since almost all the old (≥20 years of age) subgroup had high activity levels.

significance for *Eco*RV and *Fnu*4HI was borderline. The association between MAO-A allele type defined by the *Msp*I polymorphism and enzyme activity level was significant and independent of age.

Discussion

By comparison of the full-length coding sequences of cDNAs for human MAO-A from human skin fibroblasts with high and low activity, we have elucidated two single-nucleotide differences among three alleles examined. Differences at positions 941 and 1460 occur in the third base of a codon and would not alter the amino acid sequence of the encoded enzyme. Thus, in these cases, differences in activity do not result from differences in the primary structure of MAO-A. These differences do affect the presence or absence of restriction sites for *Eco*RV and *Fnu*4HI and can be evaluated by digestion of PCR-amplified cDNA or genomic fragments. This *Eco*RV polymorphism is the same as that previously described (Ozelius et al. 1988). These RFLPs and that for *Msp*I (Ozelius et al. 1989) define eight potential haplotypes at the *MAOA* locus, only three of which have been observed in over 40 individuals tested to date. These three haplotypes revealed a statistically significant association with levels of activity measured in male fibroblasts. Of 20 lines with activities <12 pmol/min/mg protein, 18 had the A allele for *MAOA;* in contrast, of 20 lines with activities ≥12 pmol/min/mg protein, only nine had this haplotype. The highest level of significance was found for the *Msp*I RFLP and was independent of donor age.

Higher levels of activity were observed in the "old" group and probably reflect the effect that aging has on MAO-A.

Levels of MAO activity in humans are undoubtedly regulated by a number of genetic and environmental factors. Genetic determinants could affect transcriptional regulation, mRNA processing and stability, translational regulation, posttranslational modifications, and protein-protein interactions. Previous studies comparing kinetic properties and the number of active molecules among lines with varying levels of activity suggested that differences in activity correlated directly with the number of active enzyme molecules (Costa et al. 1980). The differences in nucleotide sequence determined in the present study were conservative and would not affect protein structure or mRNA translatability or stability. It is still possible that variations in 5′ or 3′ noncoding regions of the message—i.e., regions not sequenced here—do affect these properties. Two forms of the human MAO-A mRNA—4.4–5.4 kb and 2.1 kb—have been described in placenta and fibroblasts, with the larger form predominating (Hsu et al. 1989; Sims et al. 1989*a;* Weyler et al. 1990). These two forms of the message differ at least in part by large variations in the length of the 3′ noncoding sequence (Hsu et al. 1989; Huang et al. 1989), variations apparently due solely to differences in polyadenylation sites. It is interesting that there is a significant lack of homology in the 3′ noncoding sequences of the two published human MAO-A cDNA sequences (Bach et al. 1988; Hsu et al. 1988). (Differences in the 5′ noncoding sequences in these reports are due to a sequence inversion in Hsu et al. 1988.) Preliminary northern blot analysis of mRNA suggests a direct correlation between levels of MAO activity and the amount of the larger form of the message, for most fibroblast lines (data not shown). The strong association between the alleles for *MAOA* and activity levels suggests that these polymorphisms mark structural differences between *MAOA* genes that regulate gene expression.

It has been difficult to assess the role of genetic variations in MAO-A activity in human disease, because it is difficult to measure the enzyme in living humans and because evaluation of activity in skin fibroblasts or metabolites in plasma or urine do not necessarily reflect activity in the nervous system. Given our finding that levels of MAO-A activity appear to be determined in large part by the *MAOA* locus itself, and given that polymorphisms at this locus can be used to mark activity states, it is now possible to assess the role of this enzyme in human disease, by evaluating the frequency of *MAOA* alleles in these and control populations. The *Eco*RV polymorphism occurs in the coding sequence and can be visualized either by Southern blot analysis of genomic DNA by using (1) either the cDNA probe HM11 or the genomic clone A2R/F2 (Ozelius et al. 1988) or (2) PCR amplification and digestion of cDNA or genomic fragments (present study). The *Msp*I polymorphism can be detected by Southern blot analysis using genomic clone A2R/D7 (Ozelius et al. 1989). The *Fnu*4HI polymorphism also occurs in the coding region and can be visualized by digestion of PCR fragments of genomic or cDNA. All these sequence variations appear to be common in the population. Allele frequencies have been determined for the *Eco*RV and *Msp*I RFLPs and give a combined PIC of .61 (Ozelius et al. 1988, 1989). These studies thus provide a means to evaluate the role of the *MAOA* gene in various neurologic and psychiatric diseases in which it has been implicated, including Parkinson disease, alcoholism, schizophrenia, and manic-depressive illness (for review, see Breakefield et al. 1980).

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